

REVIEW

Targeted drug-delivery approaches by nanoparticulate carriers in the therapy of inflammatory diseases

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Limitations in therapy induced by adverse effects due to unselective drug availability and therefore the use of potentially too high doses are a common problem. One prominent example for this dilemma are inflammatory diseases. Colloidal carriers allow one to improve delivery of drugs to the site of action and appear promising to overcome this general therapeutic drawback. Specific uptake of nanoparticles by immune-related cells in inflamed barriers offers selective drug targeting to the inflamed tissue. Here we focus on nanocarrier-based drug delivery strategies for the treatment of common inflammatory disorders like rheumatoid arthritis, multiple sclerosis, uveitis or inflammatory bowel disease.

Keywords: nanoparticles; liposomes; drug targeting; inflammatory diseases; inflamed barriers

1. INTRODUCTION

The use of nanoscaled carriers in drug delivery is expected to increase specificity of drugs and thus reduce side effects decreasing the dose of administered drugs.

Low bioavailability often limits the use of promising drug candidates and as a result drugs are potentially used in too high doses. Among others, encapsulation of biologically active molecules into nanocarriers may increase their bioavailability and may induce sustained release. The drug can be protected against degradation and its transport at the biological barrier can be altered. Besides, absorption processes can be controlled by particle properties which are thus of great relevance.

Therefore, nanoencapsulation can lead to a general change in the pharmacokinetics of the incorporated drug. The most prominent advantage of nanoscaled drug carriers over conventional drug delivery systems is the option to improve selective delivery of drugs to the site of action, so-called drug targeting which can be classified into the active and passive targeting approaches.

Passive targeting can be achieved without further integration of a specific targeting moiety on the particle surface. It occurs through different administration

routes, after intravenous (IV) application or towards an epithelium. Systemically administered particles with a hydrophobic surface are recognized by the reticuloendothelial system (RES), mainly liver and spleen, and taken up by macrophages in the bloodstream which leads to fast removal from the systemic circulation (Bazile *et al.* 1995). In general, macrophages which are produced by the spleen leading to an inflammatory response are the primary target cells in this drug targeting approach. Although targeting to those immune-related cells appears rather easy, selective targeting to organs different from liver and spleen is challenging being, however, essential for the success of a potential anti-inflammatory therapy (Kumar *et al.* 2001).

In inflammatory diseases several circumstances are known to activate the cellular immune response. In detail, an increased presence of immune-related cells like macrophages is common in the inflamed area. Tabata *et al.* (1996) have shown that microspheres and nanoparticles can be efficiently taken up by macrophages, mainly by phagocytosis (Pinto-Alphandary *et al.* 1994; figure 1). Thus, particle uptake into those immune-related cells or the disruption of the epithelium (Stein *et al.* 1998) could allow the selective accumulation of the nanocarrier-based drug delivery system in the desired area. Therefore, passive targeting can be achieved in inflammatory diseases due to immune

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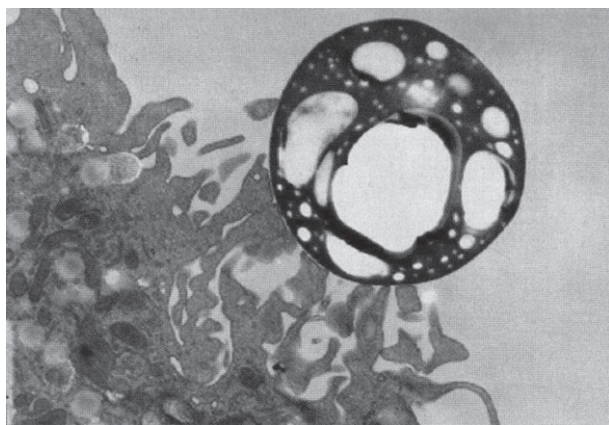


Figure 1. Adhesion of a nanoparticle on pseudopods of a macrophage imaged by transmission electron microscopy (8500 \times ; von Briesen *et al.* 2006).

response. To reach efficient uptake into those immune-related cells particle properties like size and surface charge play a key role.

Active targeting after systemic administration can be achieved by modification of the particles' surface with targeting entities. In detail, antibodies or other specific adhesion molecules are bound to the surface of particles and antigens or other specific adhesion molecule counterparts on the surface of cells can be recognized (Steeber *et al.* 2005; Simone *et al.* 2009). The drug should specifically be delivered to the site of action followed by potential intracellular uptake.

Here we give an overview on current approaches of nanocarrier-based drug delivery for the treatment of different inflammatory diseases considering IV and topical routes of application.

2. TARGETING APPROACHES AFTER IV DRUG DELIVERY

2.1. Rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic and progressive autoimmune disorder which is characterized by a chronic inflammation of the joint synovium and severe joint destruction. The precise pathophysiologic cause of RA remains uncertain. Inflammation is linked to the body's immune system attacking the tissue that lines the joints. In RA the synovial lining expands because of macrophage assembling (Van Lent *et al.* 1995; van den Berg & van Lent 1996).

A broad variety of drugs is available for the therapy of RA; however, the benefits are only temporary due to the adverse drug reactions experienced including osteoporosis, hypertension, weight gain and fluid retention, resulting in a decrease in long-term use particularly at high doses. Intra-articular injection could be one possibility to decrease exposure of the drug to unaffected tissue. This option requires repeated joint needling into a large number of joints and for this kind of treatment the size and location of the joint plays a certain role (Vanniasinghe *et al.* in press). Therefore, the development of an effective and also safe vector for targeted

delivery would be desirable. Targeting of macrophages by nanoparticulate systems has been proved as a powerful approach for the treatment of autoimmune blood disorders, diabetes, as well as RA (Barrera *et al.* 2000; Chellat *et al.* 2005; Moghimi *et al.* 2005). Additionally, accumulation of nanoparticles at sites of inflammation, such as arthritic joints (Boermann *et al.* 1997; Dams *et al.* 1999; Metselaar *et al.* 2003), has been shown which might be due to locally enhanced capillary permeability (Paleolog & Fava 1998).

Increased circulation half-life and slow release kinetics of drugs from the nanoparticulate prodrug in plasma allow a reduction in dosing frequency. IV-administered long circulating nanoparticles accumulate in organs of the RES such as spleen and liver (Storm *et al.* 1995; Gaur *et al.* 2000; Moghimi *et al.* 2001; Zhang & Mehvar 2001a,b; Brigger *et al.* 2002; Schluep *et al.* 2006). Macrophages are responsible for removing the particulates from blood and therefore they could be used as target cells for nanoparticulate RA therapy.

The macrophage-suppressive effect of liposomal encapsulated clodronate was investigated by Richards *et al.* (2001). In earlier studies clinical signs of arthritis were reduced by the uptake of liposomes by macrophages of the RES but not by macrophages located in the inflamed synovium. Accumulation in inflamed joints could have failed because liposomes were too large (Love *et al.* 1989). Clodronate was encapsulated within small unilamellar vesicles (mean size 100 nm) which were intravenously administered once to rats with chronic streptococcal cell wall-induced arthritis (Richards *et al.* 2001). The onset of arthritis was suppressed successfully by this therapy which can be associated with the elimination of not only the hepatic but also the synovial macrophages (Richards *et al.* 2001).

However, until now it has not been clear whether macrophage elimination in the joint is more important than macrophage elimination in the RES to overcome the symptoms of arthritis.

Further investigations showed that joint targeting of drugs can be achieved by IV injection of long-circulating liposomes (Metselaar *et al.* 2003).

Glucocorticoids encapsulated in liposomes can be administered systematically without eliminating macrophages but with a suppressing effect on proinflammatory functions since encapsulated drugs which eliminate macrophages from the liver and spleen strongly affect RES functioning (Camilleri *et al.* 1995). Because polyethylene glycol (PEG) is an attractive material for surface modification of nanocarriers to reduce opsonization and to prevent interactions with the RES (Bazile *et al.* 1995) PEG-coated prednisolone phosphate liposomes and the non-encapsulated drug were intravenously administered to rats with adjuvant-induced arthritis (Metselaar *et al.* 2003). Liposomal prednisolone phosphate was effective at a 10-fold lower dose. A single dose of encapsulated prednisolone phosphate led to a complete disappearance of the clinical symptoms within 2 days and remission was achieved for 6 days after treatment whereas the same dose of free drug did not decrease the paw inflammation

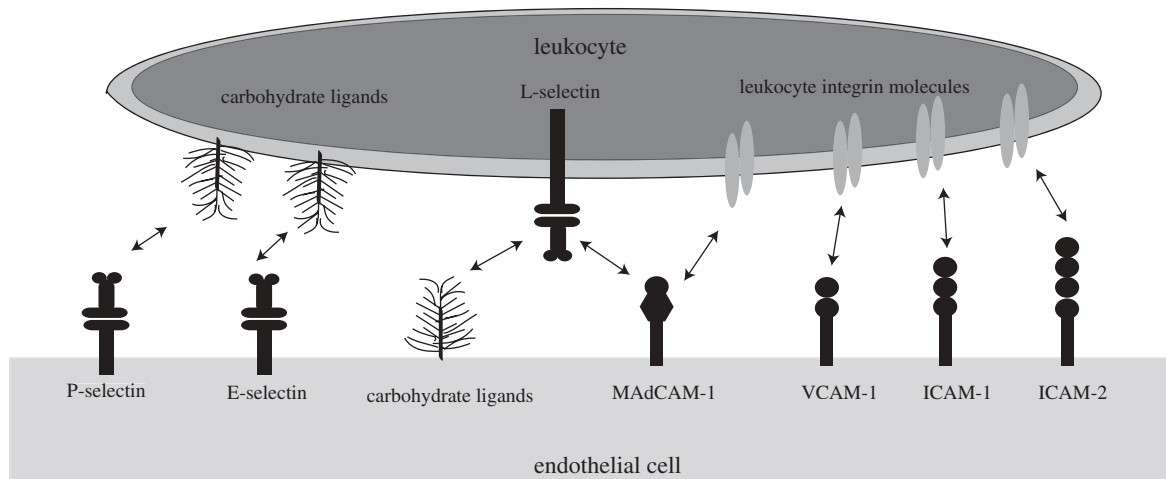


Figure 2. Cell-adhesion molecules (CAMs) are involved in the adhesion of leukocytes to endothelial cells. Before leukocytes can migrate into surrounding tissue they roll on endothelial cells and subsequently arrest to the cell surface. The initial capture and rolling of leukocytes is mediated by the interaction of selectin molecules present on both leukocytes (L-selectin) and endothelial cells (P-selectin and E-selectin) with carbohydrate ligands on the reciprocal cell-surface membrane. Also, interactions between leukocyte integrins and vascular cell adhesion molecule 1 (VCAM-1) and mucosal addressin cell adhesion molecule 1 (MAdCAM-1) on the endothelium can mediate rolling of leukocytes. Leukocyte integrin molecules are activated by chemoattractants on the endothelial surface leading to a subsequent arrest of rolling leukocytes. Integrin receptors interact with endothelial counter-receptors of the immunoglobulin superfamily intercellular adhesion molecule 1 (ICAM-1), ICAM-2, VCAM-1 and MAdCAM-1 (Ulbrich *et al.* 2003). As a consequence, nanoparticulate carriers coupled with monoclonal antibodies or synthetic compounds can easily adhere to cells involved in the extravasation process instead of leukocytes.

score. The disease-induced weight loss was only reversed by liposomal prednisolone phosphate. Also the effect of PEG coating and the size-effect of PEG-coated liposomes were evaluated *in vivo*. Small PEG-coated liposomes (90–100 nm) showed the highest therapeutic efficiency followed by non-PEG-coated liposomes whereas the large PEG-coated liposomes (450–500 nm) were only slightly effective. These results could be explained by the reduced joint targeting potential of large liposomes. On the contrary, large liposomes abolished disease-induced splenomegaly most effectively. The particle size plays a critical role in their uptake by the splenic sinusoid of animals. Normally, particles greater than 200 nm may act as splenotropic agents (Moghimi 1995; Moghimi *et al.* 2001).

Since macrophages in lymphoid organs have been shown to be involved in the development of adjuvant-induced arthritis, interest can be focused on the fact that the effect on joint inflammation is not related to spleen targeting. It appears that the better the targeting potential to inflamed joints, the stronger the therapeutic benefit. To achieve deposition of liposomes in inflamed joints, long circulation times are unavoidable. In contrast, the better the spleen uptake, the shorter the circulation half-life and consequently the joint accumulation decreases leading to less anti-inflammatory activity.

In detail, liposome uptake in hind paws of healthy rats was sevenfold smaller than uptake in arthritic rat hind paws. Also the presence of an inflammatory process as an enabling factor for PEG-coated liposome localization in the inflamed synovium has been suggested by Metselaar *et al.* (2003). Enhanced vascular permeability in the inflamed synovia seems to play a key role in selective PEG-coated liposome localization.

Besides all the above-mentioned passive targeting approaches, active targeting towards inflamed tissue has also been described. From the practical point of view, for treatment of inflammatory diseases active targeting to the endothelium can be useful since in inflammation vascular endothelium plays an important role in leucocyte recruitment and infiltration into the affected tissue (Steeber & Tedder 2000; Szekanecz & Koch 2008). Leukocytes which are strongly involved in the inflammatory process are recruited to the inflamed tissue by cell-adhesion molecules (CAMs) on the surface of the interacting cells (figure 2). The selectin family of CAMs consists of three members, namely P-selectin, E-selectin and L-selectin. Since CAMs are overexpressed in inflammatory diseases they can be used as promising targets for drug delivery to the inflammation site (Ulbrich *et al.* 2003).

Minaguchi *et al.* (2008) achieved active targeting of sialyl Lewis X (SLX) glycoliposomes at inflammation sites in collagen-antibody-induced arthritic (CAIA) mice. SLX is an E-selectin ligand expressed on the surface of leukocytes. A fluorescent substance was encapsulated into the SLX liposomes and *in vivo* fluorescent imaging showed that the signal was confined to the inflamed sections in CAIA mice in an inflammatory-dependent manner. Therapeutic efficiency after endothelial targeting with dexamethasone phosphate-containing RGD peptide liposomes was determined in an AIA rat model (Koning *et al.* 2006). The RGD peptide was covalently attached to PEG liposomes and was chosen because of its high chemical stability and high affinity for $\alpha v \beta 3$ integrin, to target angiogenic vascular endothelial cells (VECs) at sites of arthritis involvement. Finally, it could be shown that RGD-liposomes can be used as a drug delivery system which

specifically binds to VECs *in vitro* and endothelium at sites of inflammation *in vivo* with high efficiency in adjuvant-induced arthritis in rats.

2.2. Multiple sclerosis

Multiple sclerosis (MS) is an autoimmune disease associated with immune activity directed against central nervous system (CNS) antigens. It is one of the most frequent inflammatory-demyelinating diseases of the CNS. Beneath demyelination its pathological hallmarks are cellular infiltration of T-cells and macrophages, blood/brain barrier (BBB) breakdown, perivascular inflammation and the loss of axons and oligodendrocytes (Lucchinetti *et al.* 2000). The most favoured pathophysiological hypothesis includes a T-cell-dominated autoimmune reaction in association with proinflammatory macrophages.

To prevent ongoing tissue destruction with loss of oligodendrocytes, axons and neurons leading to permanent functional deficits, relapses which occur despite long-term immunotherapy are commonly treated by repeated IV injections of high-dose (pulse) glucocorticoids as a potent anti-inflammatory drug. But high-dose glucocorticoids may lead to potentially severe adverse effects and sustained influence on disability progression or protection from further relapses over time have not been delineated so far (Filippini *et al.* 2000).

Very early in MS CNS inflammatory lesion formation, molecular alterations occur in micro blood vessels at the BBB. Upon inflammatory modulation the BBB becomes permissive for the infiltration of blood compounds into the CNS parenchyma. *In vivo* investigations by new technical and methodological magnetic resonance imaging (MRI) innovations have provided new understanding of inflammatory lesion formation by distinguishing the infiltration of inflammatory cells into the brain parenchyma, in particular monocytes/macrophages of blood circulation and the molecular BBB alterations that become permissive to the passage of humoral blood compounds. First experimental MRI studies in rat animal models of MS monitored CNS infiltration of macrophages that were charged with ultra small superparamagnetic iron oxide nanoparticles in comparison to BBB leakage evidenced by gadolinium chelates (Dousset *et al.* 1999). These studies revealed that both processes are developing in distinct time and special compartments. Similar *in vivo* MRI observations distinguishing CNS macrophage infiltration and BBB leakage were made in human MS in acute relapse (Dousset *et al.* 2006; Vellinga *et al.* 2008). This approach of *in vivo* monitoring of macrophage CNS infiltration was also applied to predict CNS inflammatory disease severity (Brochet *et al.* 2006) and therapeutic evaluation (Deloire *et al.* 2004). Targeting the spleen using the passive targeting tendency mediated by macrophages may improve the therapeutic efficacy and reduce side effects (Ye *et al.* 2008). Nevertheless, complete depletion of macrophages has to be treated with care since macrophages can be activated and polarized in diverse forms (Mantovani *et al.* 2004).

Considering the plethora of macrophage activation subsets and their complex roles in immune reactions (Mantovani *et al.* 2004), there is growing evidence that macrophages play ambivalent roles in inflammation, including MS development. Indeed, various types of monocyte and macrophage activation have been identified on the basis of their functions. Using the M1/M2 nomenclature suggested by Mantovani M1 macrophages kill microorganisms and tumour cells and produce proinflammatory cytokines in large amounts. In contrast, M2 macrophages regulate inflammatory responses and adaptive type 1 immunity, scavenge debris and promote angiogenesis, tissue remodelling and repair. As a consequence, complete unselective depletion of macrophages can sensitively disturb the benefits resulting from macrophage activation like immunoregulation. For this reason, the macrophage-depleting properties of the applied drug have to be considered before developing a drug delivery system with the general aim to target cells of the RES.

Recently, the binding selectivity of charged liposomes to the spinal cord of rats with experimental autoimmune encephalomyelitis (EAE) was investigated (Cavaletti *et al.* 2009). During the early changes the BBB starts to show an enhanced permeability and also seems to be involved as mediator of neuroinflammatory processes (Harris *et al.* 1991; Brück & Stadelmann 2005). BBB targeting was determined after IV injection of positively and negatively charged liposomes. Only cationic liposomes accumulated in the spinal cord of EAE rats really fast. Thus, long circulating time intervals to obtain sufficient uptake are not necessary. Also elevated permeability is not required because of the fast uptake. In healthy rats no accumulation could be observed. Binding efficacy was proportional to disease severity and already before clinical manifestation of inflammation targeting was observed. Binding of positively charged liposomes in acute EAE could be explained by changes of BBB morphology and charge distribution similar to the binding of cationic particles to proliferating vasculature in chronic inflammation and angiogenesis. Vascular changes seem to be very early events within the inflammatory reaction in acute EAE. Using cationic vascular targeting new diagnostic and therapeutic options may become available. Cationic colloidal carriers seem to interact with the BBB in another way different from permeability increase.

To achieve ultra high tissue concentrations of glucocorticoids in the inflamed target organ and to reach a much lower systemic concentration avoiding unwanted adverse effects, long-circulating PEG-coated prednisolone liposomes were developed by Schmidt *et al.* (2003). Using an EAE rat model prednisolone liposomes were injected intravenously and prednisolone accumulation was determined after different time intervals. After 42 h, the concentration in the CNS was threefold higher compared with healthy control animals whereas spinal cord with the highest number of inflammatory lesions and BBB damage showed an accumulation 4.5-fold higher than in healthy control animals. Only in the inflamed CNS of EAE rats could an increase of

prednisolone accumulation be observed and only prednisolone liposomes clearly showed a reduction of inflammatory macrophages in the lesions. By encapsulating prednisolone higher tissue levels were achieved and the drug was delivered to the site of inflammation without a high serum concentration of the free drug. Liposomes can potentially enter the CNS directly without phagocytosis due to the high BBB permeability: 2 h after injection prednisolone concentration in the tissue increased faster than expected based on cellular diapedesis.

The efficacy of liposomal prednisolone was compared with that of liposomal methylprednisolone and to classical methylprednisolone pulse therapy in EAE rats (Linker *et al.* 2008). Prednisolone-containing PEG-coated liposomes were superior to free methylprednisolone with long-term efficacy and a sustained protection even during the second and third relapse, while methylprednisolone liposomes were found even more effective than prednisolone liposomes on the clinical course and on the level of histology. Treatment with liposomal glucocorticoids seems to be a promising new approach for relapse therapy in MS.

PEG minocycline liposomes were found to ameliorate CNS autoimmune disease (Hu *et al.* 2009). Beneath its conventional application in infectious diseases minocycline has been tested in animal models of neurodegeneration, CNS inflammation and traumatic brain injury (Yong *et al.* 2004). Treatment with IV PEG minocycline liposomes every five days showed similar effects on the clinical signs of EAE to treatment with daily intraperitoneal injections of minocycline. Accordingly, pharmacotherapy with long-circulating PEG-coated liposomes would enable the decrease of the total minocycline amount which would again minimize the risk of potential adverse effects.

3. TARGETING APPROACHES MAINLY BASED ON DRUG DELIVERY AFTER TOPICAL APPLICATION

3.1. Uveitis

Uveitis is the inflammation of the tissues forming the uveal tract, namely iris, ciliary body, choroids and contiguous structures (Lajavardi *et al.* 2007). The eye as a target organ is complex in its structure and shows a high resistance to foreign substances including drugs. One major drawback of topical formulations which are conventionally used for the treatment of eye diseases is the loss of most of the applied dose caused by the defensive mechanism of the eye. Subsequently, most of the drug is eliminated from the eye 15–20 s after application and less than 5 per cent reaches intraocular tissues after cornea penetration (Van Santvliet & Ludwig 1998; Kaur & Kanwar 2002). Due to the distinct dose increase adverse effects are caused by systemic drug absorption since most applied ophthalmic drugs are corticosteroids and non-steroidal anti-inflammatory drugs (Nagarwal *et al.* 2009).

Drug-loaded liposomes can be used for intraocular injections and so dosing frequency can be reduced. Camelo *et al.* examined the fate of fluorescent

PEG-coated liposomes after intravitreal injections in healthy rats (Camelo *et al.* 2008). Liposomes could be detected mainly in the vitreous humour, the inner retina and less at the level of the iris root and ciliary body. Macrophages and neutrophils in the conjunctiva internalized fluorescent liposomes. Lajavardi *et al.* (2007) encapsulated vasoactive intestinal peptide (VIP) in sterically stabilized PEG-coated liposomes to increase its stability in biological media and to control its release. VIP-containing liposomes were injected intravitreally to rats suffering from endotoxin-induced uveitis (EIU). It was shown that VIP was only efficient at reducing EIU when encapsulated in liposomes.

If local application is desired it is inevitable to use bioadhesive materials for effective retention in the ocular cul-de-sac. A polymer's bioadhesion properties are affected by its swelling properties, hydration time, molecular weight and degree of cross-linking (Araújo *et al.* in press). Because cornea and conjunctiva are negatively charged the mucoadhesive cationic polymer chitosan is thought to interact with extraocular structures and would represent an appropriate delivery system for incorporated drugs. The healing rate of rabbit eyes suffering from chemical injury was investigated after topical treatment with two different indomethacin chitosan nanocarriers, either nanoemulsion or nanoparticles (Badawi *et al.* 2008). Although being a potent anti-inflammatory drug the use of indomethacin as eye drops is limited because of its poor bioavailability and topical side effects. Indomethacin nanoemulsion was clearly effective in reducing corneal ulceration compared with indomethacin nanoparticles which showed moderate efficiency due to drug release studies investigated before. With nanoemulsion it was possible to achieve therapeutic concentration of indomethacin in the cornea during the study and high indomethacin levels in the inner ocular structure. A possible increase of systemic concentration and following side effects have not been investigated.

A large number of reports claim the low toxicity and good biocompatibility of chitosan following topical administration to rabbits and cell cultures (Alonso & Sanchez 2003; Diebold *et al.* 2007; Suri *et al.* 2007; Badawi *et al.* 2008). Eudragit RS100 nanoparticles were used for the encapsulation of ibuprofen and therapeutic efficiency was investigated after topical administration to rabbits after induction of eye inflammation using sodium arachidonate (SA; Bucolo *et al.* 2002). Primary signs of ocular inflammation, the protein level and the number of leukocytes in the aqueous humour were significantly reduced compared with the free drug. Also the aqueous humour drug concentration of the ibuprofen-containing nanoparticle group was higher than that of the free drug group.

Additionally, Novasorb which is a technology for targeting cornea and conjunctiva based on cationic emulsions has already entered the market. On the contrary, ulcerated tissue shows high concentrations of positively charged protein increasing the affinity to negatively charged substances. Therefore, charge interactions which possibly enhance binding of macromolecules to the inflamed tissue have to be investigated (Nagashima 1981).

Subsequently, Vega *et al.* (2006) coated flurbiprofen-loaded poly(lactic/glycolic acid) (PLGA) nanoparticles with Poloxamer 188 and obtained zeta potential values of -25 mV and a particle size of 230 nm. Poloxamer 188 was used to offer the possibility of surface modification with cationic polymers. The formulation was applied to rabbit eyes in which a mild conjunctival inflammation was received after topical instillation of SA and a very good anti-inflammatory efficacy was indicated without any signs of toxicity or irritation to ocular tissues. The anti-inflammatory response was even higher than that of commercial eye-drops (Ocuflur) containing also 0.03 per cent (w/v) of flurbiprofen. Probably these results were related to the bioadhesive properties of the polymer that increase the residence time of the colloidal system in the ocular mucosa and avoid the ocular penetration of SA. Due to physiological changes in inflamed ocular tissues the residence time of nanoparticles was higher than in healthy eyes.

The effect and localization of poly(lactic acid) (PLA) nanoparticles encapsulating betamethasone phosphate after a single IV injection to rats with experimental autoimmune uveoretinitis (EAU) was evaluated by Sakai *et al.* (2006). On confocal images it was shown that no nanoparticles could be found in the normal retina of healthy rats whereas several nanoparticles in the retina and a few nanoparticles in the outer nuclear layer could be observed 3 h after the injection in EAU rats. These nanoparticles remained in the retina of rats with EAU even 7 days after the administration. The prolonged and rapid anti-inflammatory effects achieved were equal to those of a five-times-higher dose of free betamethasone phosphate. Nanoparticles did not accumulate in the lymph nodes. Further investigation is necessary to reveal whether either repeated injections or higher doses of betamethasone phosphate enhance the anti-inflammatory effect. However, intravenously administered nanoparticles achieve ocular targeting from the endothelial side and sustained release in ocular inflammation although more than 95 per cent of the nanoparticles are trapped in the liver and spleen. As a second approach to endothelial delivery to the inflamed eye the work of Hashida *et al.* (2008) should be mentioned. Novel SLX-conjugated liposomes were loaded with dexamethasone and were injected to EAU mice. Mice which were treated with SLX liposomes showed double the concentration of dexamethasone in the eyes than those injected with the free drug solution. To confirm whether SLX liposomes target E-selectin, P-selectin or both, a binding inhibition assay was carried out which showed clearly that SLX liposomes were able to target both selectin types on the activated VECs *in vivo*.

3.2. Inflammatory bowel disease

The most common subtypes of inflammatory bowel disease (IBD) are Crohn's disease (CD) and ulcerative colitis (UC). UC and CD have many similar symptoms such as diarrhoea, bloody stools, weight loss, abdominal pain, fever and fatigue. The differences between these two diseases are the pathogenesis and the typical clinical

manifestations. In CD the inflammation is transmural extending through the bowel wall to the serosal layer and can be found throughout the small and the large intestine. In UC the innermost mucosa with no segments of normal tissue is inflamed but only in colon and rectum. Therefore, in UC rectal administration of drugs is indicated while the oral pathway should be preferred in CD due to frequent affection of ileal tissue. Normally 5-aminosalicylic acid (5-ASA) and corticosteroids are used to achieve and maintain remission. These conventional treatments afford the daily intake of high doses leading to serious adverse effects because of unspecific absorption to non-affected tissue. That is why research has been focused on the development of drug release devices which should deliver the drug specifically to the colon. Abnormal mucosal permeability caused by increased paracellular permeability has been observed in patients with IBD (Bruewer *et al.* 2003).

It could be hypothesized that the disruption of the intestinal barrier at ulcerated regions could allow the selective accumulation of the particulate carrier system in the desired area (Stein *et al.* 1998; figure 3). Besides that approach, macrophages and dendritic cells which show a strong involvement at the inflammation site in IBD (Allison *et al.* 1988; Seldenhijk *et al.* 1989) can lead to an uptake of particles into these immune-related cells.

Therefore, the size-dependent deposition of micro-particles and nanoparticles in a model of experimental colitis was examined as a first step in the development of a new selective drug delivery strategy (Lamprecht *et al.* 2001a). An increased adhesion of particles was observed at thicker mucous layers of inflamed tissue while in ulcerated regions a size dependency was shown. Targeting became more effective with smaller particles in the colitis group compared with the healthy control. It can be postulated that smaller particles can be taken up by macrophages in the inflamed areas more easily. At least, different from conventional drug delivery systems like tablets, drug targeting by nanoparticles cannot be affected by diarrhoea, a major symptom in IBD.

In the following studies it was shown that encapsulated drugs accumulate in inflamed tissue to a greater extent than when given as solution (Lamprecht *et al.* 2001b, 2005). Even after oral application of rolipram-loaded PLGA nanoparticles to rats suffering from trinitrobenzene sulphonic acid (TNBS) colitis clinical activity score decreased significantly (Lamprecht *et al.* 2001b). A possible explanation might be an accumulated deposition of nanoparticles which would retain the drug carrier in the inflamed regions of the colon for several days. For this reason the use of biodegradable polymers seems appropriate to prevent complications with long-term deposition of nanoparticles or any residual component inside the ulcerated tissue.

Tacrolimus is normally used as an immune-suppressive drug to inhibit transplantation rejection. The use of tacrolimus in IBD for the treatment of refractory UC where corticoid treatment failed (Hoshino *et al.* 1995; Aiko *et al.* 1997; Fellermann *et al.* 1998; Matsuhashi *et al.* 2000) is also common. To avoid significant adverse

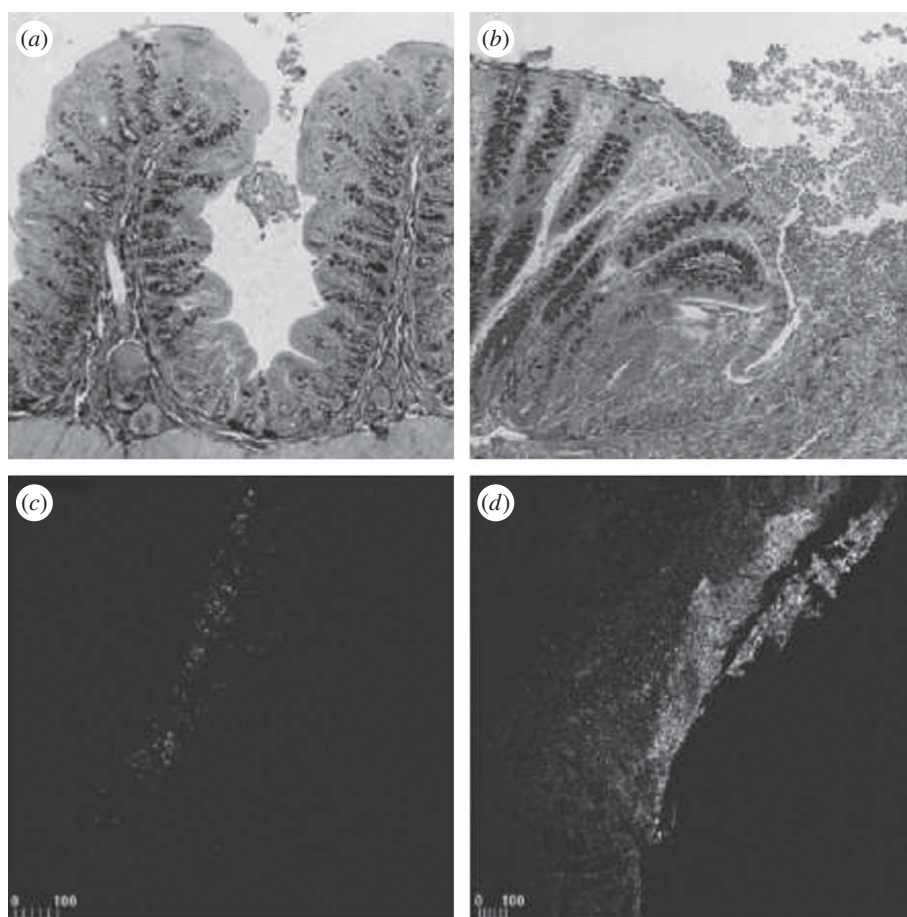


Figure 3. Microscopical images of a rat colon section through a tissue sample (a) of a healthy control group and (b) of a colitis group showing a typical ulceration in the colon. Confocal laser scanning microscopy of colon cross sections evidenced a distinct increase of particle adhesion from (c) the healthy control group to (d) the colitis group after administration of 100 nm particles (Lamprecht *et al.* 2001a).

effects like nephrotoxicity after unselective absorption (Finn 1999) tacrolimus was entrapped into poly(lactic-co-glycolic acid) nanoparticles before oral or rectal administration to rats suffering from experimental colitis (Lamprecht *et al.* 2005). An enhanced and selective drug penetration into the inflammation site was achieved, probably due to protection of the drug from efflux systems and mucosal metabolism. Also a threefold increase in drug penetration into inflamed tissue compared with healthy tissue could be recognized using nanoparticulate drug carriers.

In a study of Meissner *et al.* (2006) the therapeutic effect of either tacrolimus-loaded poly(lactic-co-glycolic acid) nanoparticles or tacrolimus-loaded pH-sensitive Eudragit P-4135F nanoparticles was compared. Both nanoparticle types showed reduced adverse effects in murine dextran sulphate sodium colitis. In contrast, free drug receiving groups showed nephrotoxicity as an adverse effect. Therapeutic efficiency was not significantly different for both therapeutic approaches. This leads to the opinion that the involved mechanisms are considered far more complex.

In the case of oral drug delivery it is important to avoid early drug leakage during the intestinal passage of nanoparticles. Otherwise the uncontrolled release of the active molecule before reaching the colon may cause adverse effects and therapeutic efficiency would

be reduced. To overcome these problems associated with uncontrolled release the targeting potential of silica nanoparticles (SiNP) in TNBS colitis after oral application was investigated (Pertuit *et al.* 2007; Moulari *et al.* 2008). Drug leakage experiments in simulated intestinal fluids were performed showing a release of Me5ASA after a lag time of around 8 h. Therefore, this time frame is sufficient enough to reach the inflamed colon after oral application without prior drug loss. It has been proved earlier that enzymatic activity including esterase activity at sites of inflammation is increased (Mork & Bundgaard 1992; Sriram *et al.* 2004).

The adhesion of SiNP to inflamed tissue was sixfold higher than in healthy control groups. Additionally, the therapeutic potential of SiNP was demonstrated by enabling a significant decrease of the necessary drug dose compared with 5ASA solution when evaluating clinical activity score and myeloperoxidase activity. Me5ASA–SiNP combine the advantages of passive drug targeting by nanoparticles and triggered release from a prodrug retaining the entrapped drug until selective accumulation in the inflamed tissue.

To study their adhesion properties charged liposomes were administered intrarectally to the colonic mucosa of healthy and colitis-induced rats (Jubeh *et al.* 2004). Cationic liposomes adhered three times

more to the healthy colonic mucosa than neutral or anionic liposomes. On the contrary, the adherence of anionic liposomes to the inflamed mucosa was twice that of either neutral or cationic liposomes. Based on these results Jubeh *et al.* (2005, 2006) encapsulated superoxide dismutase, 4-amino tempol and catalase into negatively charged liposomes. The anti-inflammatory activity of encapsulated antioxidants in rat experimental colitis was higher than that of the free molecules in every case. These results correlated to the attachment of negatively charged liposomes which consequently prolong the residence time and enhance the uptake to the inflamed mucosa. This study offers a new treatment opinion for chronic inflammation based on the novel use of antioxidants encapsulated into negatively charged liposomes.

An alternative approach in colitis therapy is the development of endothelial drug delivery strategies. Novel targeting approaches on the basis of endothelial cell adhesion molecules offer a possibility of selectively targeting the inflamed endothelium from the 'backside'. These endothelial cell adhesion molecules are upregulated in the case of inflammation. Sakhalkar *et al.* (2003) conjugated ligands to these adhesion molecules to particles made from a biodegradable block copolymer of PLA and PEG. Specific and augmented adhesion to inflamed endothelium relative to non-inflamed endothelium *in vitro* and *in vivo* was exploited. The specific targeting to vascular cell adhesion molecules-1 (VCAM-1) in a murine colitis model could also be demonstrated. Particle adhesion to the inflamed endothelium was significantly enhanced by the prepared systems and selectivity and ligand efficiency were dependent on the number of injected particles. It has to be proved whether this targeting approach is efficient enough to increase drug levels at the inflammation site to a satisfactory level to achieve a therapeutic effect and, most important, whether adverse effects can be avoided.

3.3. Gastric ulcer

The gastrointestinal adhesion behaviour of nanoparticles was examined after oral administration to mice suffering from an experimental gastric ulcer model in order to examine the influence of particle diameter on the deposition in inflamed tissue (Hasani *et al.* 2009). Highest relative adhesion was found for 50 nm particles owing to the relatively small mass of particles enabling a higher attachment to mucous layers. In gastric ulcer, similar to studies on IBD, nanoparticles selectively adhered to inflamed tissue. Inflammation leads to an enhanced mucous production in the affected tissue but the mucous layer in the stomach is thicker than in other regions. Therefore differences from ulcerated tissues in the healthy group became less visible than in colitis where alterations in mucous amount and turnover by the inflammation state have a greater impact on particle adherence.

4. GENERAL DISCUSSION

If targeting from the endothelial site is desired, it is necessary to control the opsonization process by the

use of hydrophilic polymers for surface modification or by attachment of PEG chains to biodegradable polymers such as PLA or PLGA (Soppimath *et al.* 2001). PEGylation could decrease the relatively large amount of nanoparticles trapped in the liver and spleen and therefore enhance the therapeutic efficiency even more. Protein and peptide absorption can be monitored by the hydrophilic PEG chains and cell behaviour at the polymer surface can be regulated. In conclusion, the polymeric composition (hydrophobicity, surface charge, and biodegradation profile) of the nanoparticles, any adjuvant substances and the associated drug (molecular weight, charge, localization in the nanospheres by adsorption or incorporation) have a great influence on the drug absorption, biodistribution pattern, and elimination. Even the benefit of drugs whose application has been restricted due to nephrotoxicity or stability problems can be reclassified considering their encapsulated formulations (table 1).

Also active targeting approaches have been investigated in colitis (Sakhalkar *et al.* 2003), RA (Koning *et al.* 2006; Minaguchi *et al.* 2008) and uveitis (Hashida *et al.* 2008) and the use of SLX-conjugated liposomes has proved efficient *in vivo* in both the treatment of RA and uveitis. Furthermore, many similar targets for the adhesion of ligands on the endothelial surface have already been identified (Koning *et al.* 2002) and binding efficiency has been studied *in vitro* (Everts *et al.* 2003; Banquy *et al.* 2008). Especially VCAM-1 whose expression upon endothelial cells increases in the case of inflammation has already been targeted by antibody-linked cyclopentanone prostaglandine liposomes (Homem de Bittencourt *et al.* 2007). VCAM-1 has also been used *in vivo* for liposome-mediated brain targeting (Ko *et al.* 2009) and for targeting of tumour vasculature (Gosk *et al.* 2008). However, this targeting concept needs further evaluation focusing on therapeutic efficiency in inflammatory diseases. As inflammation and cancer have several features in common and chronic inflammation predisposes an individual to cancer it can be speculated that many targeting strategies are applicable to each other. For example, tumour growth can be associated with increased endothelial permeability whereas the epithelium in inflammatory states also shows enhanced permeability. Nanocarriers can consequently target fenestrated tumour vessels (Bar *et al.* 2009) or the inflamed epithelium due to their size. Also similar adhesion molecules are overexpressed in either cancer or inflammation since tumour growth can be regarded as an inflammatory situation (Shacter & Weitzman 2002; Aggarwal *et al.* 2006). As for example angiogenesis plays a major role in RA it also presents a pathological hallmark in cancer (Carmeliet & Jain 2000). Therefore, especially RGD peptide can be used as a ligand to target angiogenic endothelial cells either at sites of inflammation (Koning *et al.* 2006) or in cancer (Carmeliet & Jain 2000). Accordingly, it will be a matter of time until targeting approaches in different inflammatory situations or in cancer will be harmonized and there is a great potential to learn from pre-existing targeting strategies in cancer

Table 1. An overview of cited drug-targeting approaches.

carrier system	size (nm)	incorporated drug	delivery strategy	disease	reference
small unilamellar vesicles	100	clodronate	endothelial	RA	Richards <i>et al.</i> (2001)
small PEG and non-PEG liposomes, large PEG liposomes	90–100 and 450–500	prednisolone	endothelial	RA	Metseelaar <i>et al.</i> (2003)
self-assembled cyclodextrin nanoparticles	27	α -methylprednisolone	endothelial	RA	Hwang <i>et al.</i> (2008)
RGD PEG liposomes	100	dexamethasone	endothelial ^a	RA	Koning <i>et al.</i> (2006)
PEG liposomes	90–100	prednisolone	endothelial	MS	Schmidt <i>et al.</i> (2003)
liposomes	90–100	prednisolone	endothelial	MS	Linker <i>et al.</i> (2008)
PEG liposomes	100–110	minocycline	endothelial	MS	Yong <i>et al.</i> (2004)
PEG liposomes	300–600	vasoactive intestinal peptide	epithelial	uveitis	Lajavardi <i>et al.</i> (2007)
chitosan nanocarriers	220–690	indomethacin	epithelial	uveitis	Badawi <i>et al.</i> (2008)
Ploxxamer 188-coated PLGA nanoparticles	232 and 277	flurbiprofen	epithelial	uveitis	Vega <i>et al.</i> (2006)
Eudragit RS100 nanoparticles	51	ibuprofen	epithelial	uveitis	Bucolo <i>et al.</i> (2002)
PLA nanoparticles	150	betamethasone	endothelial	uveitis	Sakai <i>et al.</i> (2006)
SLX liposomal nanoparticles	103	dexamethasone	endothelial ^a	uveitis	Hashida <i>et al.</i> (2008)
PLGA nanoparticles	473 and 332	rolipram	epithelial	colitis	Lamprecht <i>et al.</i> (2001b)
PLGA nanoparticles	107	tacrolimus	epithelial	colitis	Lamprecht <i>et al.</i> (2005)
PLGA nanoparticles and Eudragit P-4135F nanoparticles	455 and 469	tacrolimus	epithelial	colitis	Meissner <i>et al.</i> (2006)
silica nanoparticles	140	5-amino salicylic acid	epithelial	colitis	Moulari <i>et al.</i> (2008)
negatively charged liposomes	420, 390, 405	superoxide dismutase, 4-amino tempol, catalase	epithelial	colitis	Jubeh <i>et al.</i> (2006)

^aActive targeting approach.

(Bar *et al.* 2009; Chang *et al.* 2009; Simone *et al.* 2009). Research on nanocarrier-mediated inflammatory targeting to endothelial cells has just started and the potential of this relatively novel research topic should be evaluated carefully. VanAuker & Hood (2008) have commented that different molecules may be expressed at different times in the inflammatory process and therefore the best choice requires careful consideration of the biological background (VanAuker & Hood 2008). It was also mentioned by VanAuker & Hood that some adhesion molecules are expressed by different cell types and some have soluble forms that can enter the bloodstream leading to potential complications regarding how site specific some schemes may be. For this reason, cost–benefit analyses have to be integrated in further research work. On the contrary, passive targeting approaches are less allergenic and in general less expensive.

5. CONCLUSIONS

A lot of remarkable approaches have been developed for the therapy of inflammatory diseases. Almost all systems exhibit a higher specificity in terms of delivering the drug load to the site of action. Nanocarriers show great potential for selective drug delivery to inflamed barriers. Since the exact target cells are still unknown, further investigations are necessary in order to develop specific and disease-related adhesion mechanisms. Until now the therapeutic options appearing closest to

clinical use are based on passive targeting approaches. Other remaining problems are release control and industrial scale-up.

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